

L5 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2002 ACS  
 AN 1996:355569 CAPLUS  
 DN 125:50211  
 TI Mutational scanning of large genes by extensive PCR multiplexing and two-dimensional electrophoresis: application to the RB1 gene  
 AU Van Orsouw, Nathalie J.; Li, Daizong; van der Vlies, Pieter; Scheffer, Hans; Eng, Charis; Buys, Charles H. C. M.; Li, Frederick P.; Vijg, Jan  
 CS Mol. Genet. Section, Beth Israel Hosp. and Harvard Med. Sch., Boston, MA, 02215, USA  
 SO Hum. Mol. Genet. (1996), 5(6), 755-761  
 CODEN: HMGEE5; ISSN: 0964-6906  
 DT Journal  
 LA English  
 AB With the rapid increase in the no. of identified human disease genes, the development of accurate and cost-efficient mutation tests has become opportune. Here we present a combination of extensive PCR multiplexing and two-dimensional (2-D) DNA electrophoresis to screen for mutations in 26 exons of the retinoblastoma (RB1) tumor suppressor gene. In 2-D electrophoresis, fragments are sep'd. according to size and base pair sequence in non-denaturing and denaturing gradient gels, resp. All target fragments, designed to have optimal melting characteristics, were prep'd. in a two-step PCR (a 6-plex long-PCR pre-amplification and a subsequent 25-plex short-PCR) followed by heteroduplexing. The mixt. of PCR amplicons was then subjected to 2-D electrophoresis under a single set of exptl. conditions. With this design, 35 previously identified mutations in 18 different exons were detected in 33 bilateral retinoblastoma patients. These results suggest that 2-D electrophoresis in this format provides a generally applicable, practical and fast way to diagnose with high accuracy large genes for a broad spectrum of possible disease-causing mutations.

=> d hitseq

L5 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2002 ACS  
 IT 178305-13-0  
 RL: BUU (Biological use, unclassified); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)  
 (PCR primer; mutational scanning of large genes by extensive PCR multiplexing and two-dimensional electrophoresis and application to the RB1 gene)  
 RN 178305-13-0 CAPLUS  
 CN DNA, d(C-G-C-C-C-G-C-C-G-C-C-C-C-G-C-G-C-C-C-G-T-C-C-C-G-C-C-C-G-A-C-A-T-G-T-A-A-A-G-G-A-T-A-A-T-T-G-T) (9CI) (CA INDEX NAME)  
 NTE singlestranded  
 SEQ 1 cgcccgccgc gccccgcgcc cgtcccgccc gacatgtaaa ggataattgt

=>

BEST AVAILABLE COPY

=> d his

(FILE 'HOME' ENTERED AT 09:12:41 ON 18 APR 2002)

FILE 'CAPLUS' ENTERED AT 09:12:51 ON 18 APR 2002  
S CGACATGT/SQSN AND SQL<50

L1 FILE 'REGISTRY' ENTERED AT 09:14:55 ON 18 APR 2002  
78798 S CGACATGT/SQSN

L2 FILE 'CAPLUS' ENTERED AT 09:16:27 ON 18 APR 2002

5304 S L1

L3 0 S L2 AND SQL<50

L4 FILE 'REGISTRY' ENTERED AT 09:17:26 ON 18 APR 2002

4 S CGACATGT/SQSN AND 50/SQL

L5 FILE 'CAPLUS' ENTERED AT 09:18:03 ON 18 APR 2002

1 S L4

=>

**BEST AVAILABLE COPY**